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Interactive effects of gibberellic acid and salt stress on growth parameters and chlorophyll content in oat cultivars

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Abstract

Aim: The main objective of this study was to evaluate the possible mode of interaction between salinity and application of gibberellic acid (GA₃) and exploring how GA₃ can mitigate the harmful effects of salinity. On the basis of reduction, growth parameter and chlorophyll contents, the cultivars were categorized as tolerant, moderate and sensitive.

Methodology: To determine the effect of salinity on seed germination, seedling growth and chlorophyll contents pot experiment was carried out. Three different varieties (UPo-212, NDO-2 and UPO-94) were germinated under four different salinity levels (25, 50, 75 and 100mM). After 24 hrs., two sets of each were treated with 100 ppm of GA₃ (reused for 15 days).

Results: The results revealed that when seedling were treated with GA₃ under different salinity conditions, cv. NDO-2 and UPO-212 showed better growth and high chlorophyll content than cv. UPO-94. This study conducts that 100 ppm concentrations of GA₃ will be able to overcome the toxic effects of salt stress in seed germination, seedling growth and chlorophyll contents.

Interpretation: The study reveals that application of GA₃ is useful to mitigate salinity stress and is more effective on salt tolerant cultivars.

Introduction

Oat is a winter cereal crop which ranks sixth in world cereal production following wheat, maize, rice, barley and sorghum. It is multipurpose crop which is grown for grain, forage and crop rotation. Oat is grown in temperate zone as *rabi* crop and requires low heat and has high tolerance towards rain than other cereals, such as barley, wheat or rye (Pandey and Roy, 2011).

Salinity is one of the adverse environmental factors limiting the productivity of crop plants because most of the crop plants are sensitive to salinity. It was estimated that about 20% (45 million ha) of irrigated land, producing one-third of the world's food which is affected by salinity (Shrivastava and Kumar, 2015). Thus, it is a serious threat to agricultural productivity, especially in arid and semi-arid region (Parvaiz and Satyawati, 2008). The harmful effects of salinity can be minimized by application of various chemicals mainly Phytohormones i.e., auxins, cytokinins, brassinosteroids, Gibberellic acid (GA_3) etc. GA_3 are plant growth regulators that are also known to induce various physiological responses in plants (Chauhan *et al.*, 2015), which is most favourable for promoting and improving plant growth and photosynthetic activity (Jasmine and John, 2012). GA_3 is used to regulate plant growth by increasing cell division and cell elongation. GA_3 also maintains different biochemical properties in plants and improves grain yield (Seckin *et al.*, 2009).

Salinity affects photosynthesis by decreasing CO_2 availability as a result of diffusion limitations (Flexas *et al.*, 2007) and reduction in contents of photosynthetic pigments (Ashraf and Harris, 2013). Reduction of chlorophyll content due to salinity stress is common in salt-sensitive plant species which cause burning of leaves and other succulent parts and degradation of pigments, however, saline tolerant species that can protect themselves from such deterioration of salinity stress. Decreased level of chlorophyll has been reported in (Jallel *et al.*, 2008) in *Catheranthus roseus*; (Akca and Samsunlu, 2012) in *Juglans regia*, it has due to enzymatic chlorophyll degradation. Salinity, in general, has inhibitory effects on germination of seeds (Zhang *et al.*, 2010; Kaveh *et al.*, 2011; El-Sabagh *et al.* 2016) due to hyper-osmotic stress and hyper-ionic toxicity (Hasegawa *et al.*, 2000). Seedlings are the most vulnerable stage in the life cycle of plants and germination determines when and where seedling growth begins (Baskin and Baskin, 2014; Gioria *et al.*, 2016). Increased salinity caused a significant reduction in germination percentage, germination rate, root and shoot length and fresh weight of root and shoot (El-Shaieny, 2015).

Considering the trends of growth reduction due to salinity and progressive impact of exogenous GA_3 application on different morphological, physiological and biochemical activities, it can be stated that application of GA_3 is useful to mitigate salinity stress and its effectiveness is more dynamic to salt tolerant cultivar

(Misratia *et al.*, 2015). The evidence for hormone involvement comes from correlation of hormone concentration with specific development stages, effects of applied hormones and relationship of hormones to metabolic activities. The application of GA_3 increases the plant growth by attributing the fact that they increase the amino acid content in embryo and induces the syntheses of hydrolytic enzyme required for digestion of endospermic starch when seeds renew growth at germination (Jasmine and John, 2012).

The aim of this research was studying GA_3 effect on seed germination, early growth and chlorophyll content of oat cultivars under salinity stress.

Materials and Methods

To study the response of oats to GA_3 during salt stress a pot experiment was carried out. Sand culture method was applied to test the interactive effects of salinity and GA_3 on oat cultivars. Three oat cultivars NDO-2 from Narendra Dev University of Agriculture & Technology, Faizabad (U.P.), UPO-212 and UPO-94 were procured from G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand) for performing the experiment.

The germination count was recorded at alternate day until germination stopped. The length, fresh weight of roots and shoots were recorded at 15 days (approximately) after sowing. Shoot and root length were recorded by taking average of three plants in each treatment. Plants harvested from each treatment were washed with water to eliminate the contamination. Water was soaked with blotting paper. Root and shoot of plant selected from each treatment was separated carefully and their fresh weight was measured with the help of an electronic balance. The germination percentage was calculated.

Chlorophyll content in fresh leaves was determined at seedling stage (15 days after germination) to assess interactive effect of salinity and GA_3 on chlorophyll contents. The chlorophyll content was estimated following the method of Arnon (1949).

Statistical analysis: Data presented is mean values from two independent experiments (with and without GA_3) each with three replicates. Experimental data were analyzed with the SPSS Statistical Computer Package (SPSS for WINDOWS, Standard Version 20.0) with PSiT (Paired Sample t-Test) at $P = 0.05\%$ level.

Results and Discussion

The interactive effects of salinity and GA_3 on seed germination of oat cvs. (NDO-2, UPO-212 and UPO-94) is shown in Fig.1. The statistical analysis (Paired sample t-test) indicates that seed germination was significantly affected in cvs. NDO-2 and UPO-94, except in UPO-212 with respect to all salinity levels

(Table 1). Salinity, in general case, has inhibitory effect on germination of seeds (Akbarimoghaddam *et al.*, 2011) in wheat and oat species (Zhang *et al.*, 2013). The cv. UPO-212 exhibited maximum percentage germination at all levels of salinity including control, but minimum reduction was achieved by cv. NDO-2 in both the case. At high salinity levels (75 and 100 mM), higher reduction (46.86 to 56.36%) was achieved in cv. UPO-94 and least reduction (26.64 to 36.02%) in NDO-2 followed by UPO-212 (37.90 to 47.33%). Present results indicate that the percentage germination reduced significantly with increase in saline concentration in oat cultivars. Similar results were reported earlier by Kumar *et al.* (2014); Chauhan *et al.* (2016) and Benlioglu and Ozkan (2016) in oats. Exogenous application of GA₃ through foliar or presoaking seed is good option to alleviate the adverse effect of salinity stress on crops (Ashraf *et al.*, 2008). The increased germination percentage in GA₃ treated seeds might be attributed to fact that the GA₃ helps in breaking the seed dormancy which results in early and enhanced seed germination due to the diffusion of endogenous auxin and gibberellins like substances (Gurung *et al.*, 2014). GA₃ reduces the harmful effects of salinity

on germination. When seeds were treated with GA₃, cv. UPO-94 exhibited maximum reduction (17.92 to 48.13%) while minimum reduction (7.20 to 26.87%) was found in cv. NDO-2 respectively at all salinity levels (25 to 100 mM). Tolerant cv. NDO-2 revealed higher germination percentage while sensitive cultivar UPO-94 showed low germination under different salinity levels (with and without GA₃). GA₃ has been reported to increase germination percentage and overcome the preventive effects of the salt stress on germination (Ghodrat and Roustae (2012). A few studies have, however, demonstrated the ability of foliar pretreatment with GA₃ to overcome effects of salt stress (Patel and Mankad (2014).

Effect of salinity and GA₃ on length of shoot and root showed decreasing trend with increasing levels of salinity. However, application of GA₃ alleviated the negative effects of salinity since the above mentioned characters were significantly increased. The inhibitory effect was more pronounced in root than shoot in both groups (Fig. 2 a, b). Under salinity treatment, higher reduction (18.17 to 51.01% and 23.52 to 57.76%) was observed in variety UPO-94 and lesser reduction (12.90 to 37.60% and 15.80

Table 1 : Effect of GA₃ and salinity stress on different cultivars of oat

Cultivars	Mean difference (with GA ₃ without GA ₃)				
	Germination %	Shoot length (cm)	Root length (cm)	Fresh weight of shoot (mg)	Fresh weight of root (mg)
NDO-2	9.23 ± 2.09*	6.56 ± 0.32*	3.77 ± 0.14*	13.13 ± 6.96*	10.61 ± 4.36*
UPO-212	5.29 ± 4.85 ^{ns}	5.05 ± 0.13*	2.53 ± 0.10*	10.64 ± 4.06*	8.03 ± 3.09*
UPO-94	4.46 ± 0.84*	3.19 ± 0.41*	1.39 ± 0.15*	6.14 ± 3.03*	6.87 ± 2.04 ^{ns}

Values are mean of four replicates ± SD; Data subjected to paired sample 't' test; (*) significant at 0.05 level; (ns) non-significant

Fig. 1 : Interactive effects of salinity and GA₃ on germination % on three cultivars of oat; (Without GA₃ all treatment (25, 50, 75 and 100 mM NaCl concentrations) was compared with control; GA₃ + salt treatment was compared with NaCl treatment. Application of GA₃ significantly promoted the germination percentage under saline conditions)

Fig. 2 : Interactive effects of salinity and GA₃ on (a) shoot length and (b) root length on three cultivars of oat ; (Without GA₃, all salt treatment (25, 50, 75 and 100 mM) was compared with control; GA₃ + salt treatment was compared with NaCl treatment. Application of GA₃ significantly promoted the length of shoot and root under saline conditions)

to 42.82%) in cv. NDO-2 was recorded respectively in shoot and root length at 25 to 100 mM NaCl concentrations. The present results indicate that shoot and root length in different oat cultivars reduced significantly with increasing salinity levels from 25 to 100 mM. These results are in agreement with those of Akbarimoghaddam *et al.* (2011) who showed that NaCl salinity caused a marked reduction in shoot and root lengths. Salt stress reduced the ability of plants to absorb water which leads to reduction in growth (Nawaz *et al.*, 2010). The inhibitory effect of salinity on growth rate and development of seedlings was reported

by Rajput *et al.* (2015) in castor bean. Salt stress reduced the ability of plants to absorb water which leads to reduction in growth (Nawaz *et al.*, 2010). Applications of GA₃ significantly promoted the length of shoot and root in all the cultivars of oat at all salinity treatments. Tolerant cultivar NDO-2 showed the maximum height (25.50 to 19.10 cm and 17.60 to 11.99 cm) and minimum height was found in sensitive cv. UPO-94 ranging from 17.24 to 10.66 cm and 11.13 to 5.75 cm in shoot and root length respectively, at varying salinity levels (25 to 100 mM) including control. GA₃ treated oat plants showed an increase in tolerance to salt

Table 2 : Effects of gibberellic acid on chlorophyll contents a and b (mg g⁻¹ f.wt.) in three oat cultivars under salinity stress

Cultivars	Mean difference (with GA ₃ without GA ₃)		
	Chlorophyll 'a'	Chlorophyll 'b'	Chlorophyll a + b
NDO-2	0.12 ± 0.007*	0.05 ± 0.005*	0.17 ± 0.011*
UPO-212	0.08 ± 0.005*	0.03 ± 0.004*	0.11 ± 0.005*
UPO-94	0.06 ± 0.014*	0.02 ± 0.005 ^{ns}	0.08 ± 0.009*

Values are mean of four replicates ± SD; Data subjected to paired sample 't' test; (*) significant at 0.05 level; (^{ns}) non-significant

Fig. 3 : Interactive effects of salinity and GA₃ on (a) fresh weight of shoot and (b) fresh weight of root on three cultivars of oat; (Without GA₃ all salt treatment (25, 50, 75 and 100 mM) was compared with control; GA₃ + salt treatment was compared with NaCl treatment. Application of GA₃ significantly promoted the fresh weight of shoot and root under saline conditions)

treatment. This increase in salt tolerance was reflected in growth. Length of shoot and root was increased when compared with non-treated plants. The effect of GA₃ was also consistent over salinity levels and gibberellic acid alleviated the harmful effects of salinity and enhanced plant height. Javid *et al.*, (2011) and Shaddad *et al.*,

(2013) also reported a similar increase in the growth of shoot and root of soybean plants in response to GA₃ treatment. Bejaoui (1985) has reported that the effects of exogenously applied GA₃ in alleviation of salt stress may be caused by activation of special enzymes which participate in RNA and protein synthesis.

Fig. 4 : Interactive effects of GA₃ and salt stress on (a) Chl a; (b) Chl. b and (c) Chl. (a + b) on three cultivars of oat; (Without GA₃, all salt treatment (25, 50, 75 and 100 mM) was compared with control; GA₃ + salt treatment was compared with NaCl treatment. Exogenous application of GA₃ significantly increased the chlorophyll contents under salt concentrations (25 to 100 mM). Cv. NDO-2 performed better under salinity stress when application of GA₃ was given.)

Interactive effects of GA₃ and NaCl salinity stress on fresh weight of shoots and roots is shown in fig. 3 (a, b). Data indicated that cv. NDO-2 and UPO-212 statistically significant paired differences (with and without GA₃) in fresh weight of shoot and root, respectively, at all levels of salinity including control (Table. 1). The highest fresh weight of shoot and root were obtained in cv. NDO-2 and lowest in cultivar UPO-94 at all salinity levels. At 25 to 100 mM saline concentrations, the fresh weight of shoot and root was highly reduced in cv. UPO-94. It ranged from 20.88 to 56.29% and 31.25 to 63.86% in shoot and root respectively. Cv. NDO-2 showed lower reduction (14.13 to 51.34% and 20.30 to 56.36% followed by UPO-212 (18.61 to 26.15% and 25.59 to 50.24%) in fresh weight of shoot and root at these salinity levels. The fresh weight of shoot and root also showed a considerable decrease at higher salinity levels. However, roots were more sensitive to salinity as compared to shoots. These results are similar to those reported by Agarwal *et al.* (2015), in soybean. Reduction in fresh biomass at higher concentration might be due to poor absorption of water from the growth medium due to physiological drought (Nedjimi *et al.*, 2006; Seckin *et al.*, 2009). Fresh weights were decreased with an increase in the level sodium chloride as a result of reduced photosynthesis and membrane stability (Mozafariyan *et al.*, 2013). Under GA₃ treatment, cv. UPO-94 showed greater reduction (18.11 to 48.78% and 26.03 to 54.93%) while lesser reduction (11.76 to 33.06% and 17.02 to 36.39%) was achieved in cv. NDO-2 followed by UPO-212 (14.54 to 38.79% and 21.89 to 44.75%) on fresh weight of shoot and root, respectively, at all salinity levels (25 to 100 mM). In oat water-deficit and salt treatments also caused reduction in seedling fresh weight, which is similar to several reports (Agarwal and Panday, 2004; Ramezani *et al.*, 2011). Exogenous application of GA₃ was promoted, generally growth parameters (fresh weight of shoot and root) of oat plants, thus alleviating to some extent the oppressive effect of salinity (Abd El-Samad and Shaddad, 2013).

The effect of GA₃ on chlorophyll contents of oat cultivars under different saline concentrations is shown in Fig. 4 (a, b, c). Chlorophyll *a* and *b* was higher in tolerant cv. NDO-2 (0.60 to 0.33 and 0.19 to 0.12 mg g⁻¹ f.wt.) and lower in sensitive cv. UPO-94 (0.37 to 0.14 and 0.12 to 0.05 mg g⁻¹ f.wt.) at all salinity levels as compared to control. However, chlorophyll *b* was more sensitive than chlorophyll *a*. The maximum chlorophyll (*a* + *b*) was observed in NDO-2 and minimum in UPO-94 at 25 to 100 mM. In this study, chlorophyll contents were significantly decreased with the increase of salinity levels in all cultivars of oat. Consistent with these results, Khalid and Cai (2011); Celik and Atak (2012) found an inhibition in chlorophyll bio synthesis in sorghum due to salt stress. The decrease may be due to the formation of proteolytic enzymes such as chlorophyllase, which is responsible for chlorophyll degradation (Sabater and Rodriguez, 1978). Application of GA₃ improved the chlorophyll levels in salinity-stressed plants of oat. GA₃ treated plants exhibited higher values of chlorophyll contents in tolerant cultivar NDO-2 as compared to

other cultivars. Similarly, Datta *et al.*, (2009) observed that GA₃ treatment increased chlorophyll contents as well as the rate of photosynthesis in soybean plants. GA₃ treatment alleviated the negative effects of salinity on the morphological traits and physiological attributes such as chlorophyll content and stomatal conductance (Misratia *et al.*, 2013 and Nikee *et al.*, 2014).

It is concluded from the present investigation that exogenous application of GA₃ might counteract the adverse effect of salinity. NDO-2 and UPO-212 proved to have better growth and high chlorophyll contents as compared to UPO-94 when seedling were treated with GA₃ under different salinity conditions. On the other hand, the inhibitory and deleterious effects of salinity (the plants irrigated with saline water) seemed to be decreased by application of GA₃.

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